

## Distribution of photoperiod-insensitive allele *Ppd-A1a* and its effect on heading time in Japanese wheat cultivars

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The *Ppd-A1* genotype of 240 Japanese wheat cultivars and 40 foreign cultivars was determined using a PCR-based method. Among Japanese cultivars, only 12 cultivars, all of which were Hokkaido winter wheat, carried the *Ppd-A1a* allele, while this allele was not found in Hokkaido spring wheat cultivars or Tohoku-Kyushu cultivars. Cultivars with a photoperiod-insensitive allele headed 6.9–9.8 days earlier in Kanto and 2.5 days earlier in Hokkaido than photoperiod-sensitive cultivars. The lower effect of photoperiod-insensitive alleles observed in Hokkaido could be due to the longer day-length at the spike formation stage compared with that in Kanto. Pedigree analysis showed that ‘Purple Straw’ and ‘Tohoku 118’ were donors of *Ppd-A1a* and *Ppd-D1a* in Hokkaido wheat cultivars, respectively. Wheat cultivars recently developed in Hokkaido carry photoperiod-insensitive alleles at a high frequency. For efficient utilization of *Ppd-1* alleles in the Hokkaido wheat-breeding program, the effect of *Ppd-1* on growth pattern and grain yield should be investigated. *Ppd-A1a* may be useful as a unique gene source for fine tuning the heading time in the Tohoku-Kyushu region since the effect of *Ppd-A1a* on photoperiod insensitivity appears to differ from the effect of *Ppd-B1a* and *Ppd-D1a*.

**Key Words:** wheat, photoperiod response gene, *Ppd-A1*, heading time, Japanese wheat cultivars.

### Introduction

The heading time of wheat (*Triticum aestivum* L.) is a complex characteristic controlled by narrow-sense earliness (also termed earliness *per se*) and is modified by vernalization and photoperiod responses (Kato and Yamashita 1991, Yasuda and Shimoyama 1965). It has been also reported that the photoperiod response is the major determinant of the earliness of autumn-sown wheat in central and southwestern Japan (Tanio *et al.* 2006, Yasuda and Shimoyama 1965, Yoshida *et al.* 1983) and heading time is independent of the *Vrn* genotype controlling the vernalization response (Fujita *et al.* 1995, Kato and Yamashita 1991, Tanio *et al.* 2005).

The photoperiod response is mainly controlled by three

major genes, *Ppd-D1* (previously designated *Ppd1*), *Ppd-B1* (*Ppd2*) and *Ppd-A1* (*Ppd3*), which are located in a homoeologous group of two chromosomes (Scarth and Law 1983, 1984, Welsh *et al.* 1973). The barley homoeologue *Ppd-H1* was identified as a member of the pseudo-response regulator (*PRR*) gene family (Turner *et al.* 2005). The orthologous *PRR* genes of the A, B and D genomes have been isolated from wheat bacterial artificial chromosome (BAC) libraries and sequence analyses revealed that the photoperiod-insensitive *Ppd-D1a* allele is associated with a 2089-bp deletion in the 5' upstream region (Beales *et al.* 2007). Recent data showed that the photoperiod-insensitive *Ppd-A1a* and *Ppd-B1a* alleles are associated with a 1085-bp deletion and 308-bp insertion, respectively, both in the 5' upstream region (Nishida *et al.* 2012). Deletion of *Ppd-A1a* shares a common region with the deletion of *Ppd-D1a*, while insertion of *Ppd-B1a* interrupts the common region.

Previous studies have investigated the geographical

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distribution of *Ppd-D1a*. Based on the sequence polymorphism among *Ppd-D1* alleles reported by Beales *et al.* (2007), Yang *et al.* (2009) determined the *Ppd-D1* genotype of Chinese wheat landraces and found that the frequency of the *Ppd-D1a* allele varies among regions, even within China. The *Ppd-D1a* allele was not found in northern China, but was frequently observed in southeastern China. Most of the European wheat cultivars with photoperiod insensitivity likely carry *Ppd-D1a* derived from 'Akakomugi' (Worland 1996). This assumption is supported by Guo *et al.* (2010), who showed that most Italian wheat cultivars carry *Ppd-D1a*. However, little is known regarding the distribution of the photoperiod-insensitive alleles *Ppd-A1* and *Ppd-B1* since the critical sequence polymorphism among alleles was not detected until recently by Nishida *et al.* (2012).

For Japanese wheat cultivars, most cultivars in the Tohoku-Kyushu region carried *Ppd-D1a* and extra-early cultivars in southwestern Japan carried *Ppd-B1a* and *Ppd-D1a* (Seki *et al.* 2011). *Ppd-B1a* showed a stronger effect on accelerating heading than *Ppd-D1a* (Seki *et al.* 2011, Tanio *et al.* 2007). Therefore, it was suggested that introduction of the *Ppd-B1a* allele enabled the breeding of early maturity wheat cultivars required to avoid preharvest sprouting and Fusarium head blight during the rainy season. The photoperiod-insensitive allele *Ppd-D1a* has also been found in Hokkaido wheat cultivars, although its effect on heading time was insignificant against the genetic background of Hokkaido wheat cultivars (Seki *et al.* 2011). In addition to these two alleles, another photoperiod-insensitive allele *Ppd-A1a* was found in a Hokkaido cultivar 'Chihokukomugi' by Nishida *et al.* (2012). They compared the heading time among DH lines differing in the *Ppd-1* genotype and showed that the effect of *Ppd-A1a* was weaker than that of *Ppd-B1a* or *Ppd-D1a*. However, since an insensitive allele of *Ppd-A1* had not been previously reported, very little is known regarding the effect of *Ppd-A1a* and its distribution in Japanese wheat cultivars.

In this study, the *Ppd-A1* genotype of Japanese wheat cultivars was determined using a PCR-based method to detect the deletion in the upstream region of this gene. Thereafter, the *Ppd-A1* genotype of wheat cultivars and breeding lines present in the pedigree of Hokkaido winter wheat cultivars was determined to reveal the origin of the *Ppd-A1a* allele and to discuss the effect of *Ppd-A1a* and *Ppd-D1a* alleles on heading time in Hokkaido wheat cultivars.

## Materials and Methods

A total of 280 wheat cultivars, consisting of Japanese commercial cultivars (164 cultivars), Japanese breeding lines (52 cultivars), Japanese landraces (24 cultivars) and foreign cultivars introduced for breeding (40 cultivars), were used in the present study. The geographical origin of these cultivars is summarized in Table 1.

For DNA extraction, all wheat genotypes were grown in a growth chamber maintained at 20°C under a natural photo-

period. Genomic DNA was extracted from 2-week-old seedlings using a modified CTAB method (Murray and Thompson 1980).

The *Ppd-1* genotypes were determined using PCR-based methods with primer sets designed to identify the deletion of 1085 bp in the upstream region of *Ppd-A1a*, the insertion of 308 bp in the upstream region of *Ppd-B1a* (Nishida *et al.* 2012), or the deletion of 2089 bp in the upstream region of *Ppd-D1a* (Beales *et al.* 2007). Three primers, namely TaPpd-A1prodeLF1, TaPpd-A1prodeLR3 and TaPpd-A1prodeLR2 (developed by Nishida *et al.* 2012), were used for *Ppd-A1* and two primers, namely TaPpd-B1proF1 and TaPpd-B1int1R1 (developed by Nishida *et al.* 2012), were used for *Ppd-B1*. Three primers, namely Ppd-D1\_F1, Ppd-D1\_R1 and Ppd-D1\_R2 (developed by Beales *et al.* 2007), were used for *Ppd-D1*. The nucleotide sequence of each primer is shown in Table 2.

For the analysis of *Ppd-A1* and *Ppd-D1*, PCR amplification was performed in a 5 µl mixture containing 10 ng genomic DNA, 0.5 µl 10 × *Ex Taq* buffer (TaKaRa, Shiga, Japan; 20 mM Tris-HCl at pH 8.0, 100 mM KCl, 20 mM Mg<sup>2+</sup>), 0.2 mM dNTP, 0.2 µM of each primer and 0.125 U *Ex Taq* Hot Start Version (TaKaRa). The PCR cycle for *Ppd-A1* was as follows: an initial denaturing step at 98°C for 30 sec; 35 PCR cycles at 98°C for 10 sec, 57°C for 30 sec and 72°C for 30 sec and a final extension step at 72°C for 2 min. The PCR cycle for *Ppd-D1* was as follows: an initial denaturing step at 98°C for 30 sec; 35 PCR cycles at 98°C for 10 sec, 54°C for 1 min and 72°C for 30 sec and a final extension step at 72°C for 2 min. For the analysis of *Ppd-B1*, PCR amplification was performed in a 5 µl mixture containing 10 ng genomic DNA, 0.5 µl 10 × *Pyrobest* buffer (TaKaRa; 50 mM Tris-HCl at pH 8.2, 10 mM Mg<sup>2+</sup>), 0.2 mM dNTP, 0.2 µM of each primer and 0.125 U *Pyrobest* DNA polymerase (TaKaRa). The PCR cycle was as follows: an initial denaturing step at 98°C for 30 sec; 35 PCR cycles at 98°C for 10 sec, 64°C for 1 min and 72°C for 30 sec and a final extension step at 72°C for 2 min. Amplification reactions were conducted using a GeneAmp PCR System 9700 thermal cycler (Applied Biosystems, Foster City, CA, USA). PCR products were separated by electrophoresis on a 13% polyacrylamide gel. Electrophoresis and polymorphism detection were based on the description by Hori *et al.* (2003). For 258 cultivars, the *Ppd-B1* and *Ppd-D1* genotypes were analyzed in our previous study (Seki *et al.* 2011) and their genotype data were used in this study.

Among them, 23 wheat cultivars and breeding lines appeared in the pedigree of 'Kitahonami', the latest registered winter wheat cultivar cultivated in Hokkaido and were grown in a field at the NARO Institute of Crop Science (Tsukuba, Ibaraki, 36°01'N, 140°06'E) in the Kanto region and the HRO Kitami Agricultural Experiment Station (Kunneppu, Hokkaido, 43°47'N, 143°42'E) in the Hokkaido region of Japan. The details are summarized in Table 3.

In Tsukuba, they were sown on November 6 and November 5 in the 2008/2009 and 2009/2010 wheat-growing

**Table 1.** Geographical origin of 280 wheat cultivars and geographical distribution of *Ppd-1* alleles

| Area of origin          | Total number<br>of cultivars | <i>Ppd-A1</i>  |                | <i>Ppd-B1</i>  |                | <i>Ppd-D1</i>  |                |
|-------------------------|------------------------------|----------------|----------------|----------------|----------------|----------------|----------------|
|                         |                              | <i>Ppd-A1a</i> | <i>Ppd-A1b</i> | <i>Ppd-B1a</i> | <i>Ppd-B1b</i> | <i>Ppd-D1a</i> | <i>Ppd-D1b</i> |
| Hokkaido (Winter wheat) | 29                           | 12             | 17             | 0              | 29             | 7              | 22             |
| Hokkaido (Spring wheat) | 10                           | 0              | 10             | 0              | 10             | 1 <sup>f</sup> | 9              |
| Tohoku, Hokuriku        | 47                           | 0              | 47             | 0              | 47             | 43             | 4 <sup>g</sup> |
| Kanto, Tokai            | 63                           | 0              | 63             | 2 <sup>b</sup> | 61             | 63             | 0              |
| Kinki, Chugoku, Shikoku | 43                           | 0              | 43             | 6 <sup>c</sup> | 37             | 42             | 1 <sup>h</sup> |
| Kyushu                  | 48                           | 0              | 48             | 2 <sup>d</sup> | 46             | 48             | 0              |
| Foreign cultivars       | 40                           | 2 <sup>a</sup> | 38             | 1 <sup>e</sup> | 39             | 18             | 22             |
| Total                   | 280                          | 14             | 266            | 11             | 269            | 222            | 58             |

<sup>a</sup> Carrier of *Ppd-A1a*; ‘Purcam (U-11)’ and ‘Purple Straw’.<sup>b</sup> Carrier of *Ppd-B1a*; ‘Konosu 4’ and ‘Shiroboro 21’.<sup>c</sup> Carrier of *Ppd-B1a*; ‘Chugoku 55’, ‘Chugoku 81’, ‘Chugoku 91’, ‘Chugoku 98’, ‘Chugoku 114’ and ‘Fukuwasekomugi’.<sup>d</sup> Carrier of *Ppd-B1a*; ‘Sakigakekomugi’ and ‘Abukumawase’.<sup>e</sup> Carrier of *Ppd-B1a*; ‘Tapdongmil’.<sup>f</sup> Carrier of *Ppd-D1a*; ‘OS-21’.<sup>g</sup> Carrier of *Ppd-D1b*; ‘Fultz Daruma’, ‘Norin 6’, ‘Norin 24’ and ‘Norin 38’.<sup>h</sup> Carrier of *Ppd-D1b*; ‘Eshima’.**Table 2.** Diagnostic PCR primers used to determine the *Ppd-1* genotypes

| Locus         | Primer name               | Sequence (5'→3')     |
|---------------|---------------------------|----------------------|
| <i>Ppd-A1</i> | Ta <i>Ppd-A1</i> prodelF1 | CGTACTCCCTCCGTTCTTT  |
|               | Ta <i>Ppd-A1</i> prodelR3 | AATTACGGGGACCAAATACC |
|               | Ta <i>Ppd-A1</i> prodelR2 | GTTGGGGTCGTTGGTGGTG  |
| <i>Ppd-B1</i> | Ta <i>Ppd-B1</i> proF1    | ACACTAGGGCTGGTCGAAGA |
|               | Ta <i>Ppd-B1</i> intR1    | CCGAGCCAGTGCAAATTAAC |
| <i>Ppd-D1</i> | Ta <i>Ppd-D1</i> _F1      | ACGCCTCCCACTACACTG   |
|               | Ta <i>Ppd-D1</i> _R1      | TGTTGGTCAAACAGAGAGC  |
|               | Ta <i>Ppd-D1</i> _R2      | CACTGGTGGTAGCTGAGATT |

seasons, respectively. Each experimental plot consisted of a single 1.0-m-long row; the planting distance was 70 cm between rows and 8.5 cm between plants. At Kunneppu, they were sown on September 21, September 20 and September 19 for the 2007/2008, 2008/2009 and 2009/2010 wheat-growing seasons, respectively. Each experimental plot consisted of six 4.5-m-long rows; the planting distance was 20 cm between rows and 255 seeds per square meter. Heading time was recorded when the tip of the first ear emerged from the flag leaf sheath in half of the plants for each cultivar.

Heading date data were analyzed using statistical software (SPSS Ver. 18.0 J for Windows; SPSS Japan Inc.). The effective day-length in Tsukuba and Kunneppu was calculated according to methods presented by Gotoh (1977), who stated that the effective day-length should include predawn with light intensity over 20 lux and twilight with light intensity over 10 lux, which were 26 and 23 min in Fukuyama (34°30'N), respectively. The calculation formula was as follows:

$$\text{(effective day-length in minutes at } \theta \text{ degrees latitude)} \\ = (\text{astronomical day-length}) + 49 (\cos 34^{\circ}30' / \cos \theta).$$

## Results

Expected PCR product sizes, i.e., 338 bp from *Ppd-A1a* or 299 bp from *Ppd-A1b*, a photoperiod-sensitive allele without deletion of 1085 bp, were successfully amplified by multiplex PCR in all of the cultivars tested (Supplemental Fig. 1). For *Ppd-B1*, 1600 bp from *Ppd-B1a* or 1292 bp from *Ppd-B1b*, a photoperiod-sensitive allele without an insertion of 308 bp and for *Ppd-D1*, 288 bp from *Ppd-D1a* or 415 bp from *Ppd-D1b*, a photoperiod-sensitive allele without deletion of 2089 bp, were successfully amplified in all of the cultivars tested. Among 280 cultivars, only 14 cultivars (5.0%) carried the *Ppd-A1a* allele (Table 1), i.e., 12 Hokkaido winter wheat cultivars and two foreign cultivars, ‘Purcam (U-11)’ and ‘Purple Straw’ (Table 1 and Supplemental Fig. 2). The *Ppd-A1a* allele was not found in Hokkaido spring wheat cultivars or Tohoku-Kyushu cultivars. Most of the cultivars in the Tohoku-Kyushu region and eight cultivars in the Hokkaido region carried the photoperiod-insensitive allele *Ppd-D1a* and 11 cultivars, including three extra-early commercial cultivars, carried the two photoperiod-insensitive alleles *Ppd-B1a* and *Ppd-D1a*, as previously reported (Seki *et al.* 2011). The other genotypes with two or three photoperiod-insensitive alleles, i.e., the *Ppd-A1a/Ppd-B1a/Ppd-D1b*, *Ppd-A1a/Ppd-B1b/Ppd-D1a* and *Ppd-A1a/Ppd-B1a/Ppd-D1a* genotypes, were not found in this study.

Among the 23 genotypes in the pedigree of ‘Kitahonami’, 10 carried *Ppd-A1a* and five carried *Ppd-D1a* as a photoperiod-insensitive allele, while the others did not carry insensitive alleles of *Ppd-1* (Table 3). The heading date examined in Tsukuba and Kunneppu was compared among three *Ppd-1* genotypes, namely *Ppd-A1a/Ppd-B1b/Ppd-D1b*, *Ppd-A1b/Ppd-B1b/Ppd-D1a* and *Ppd-A1b/Ppd-B1b/Ppd-D1b* (Table 4). The genotypic difference of heading date was significant at the 0.1% level in Tsukuba and 5% level in Kunneppu. In Tsukuba, the average heading date of

**Table 3.** Wheat cultivars tested at Tsukuba and Kunneppu and their *Ppd-1* genotype and heading date

|                 | <i>Ppd-1</i> genotype     |                           |                           | Heading date         |           |         |                       |           |           |         |
|-----------------|---------------------------|---------------------------|---------------------------|----------------------|-----------|---------|-----------------------|-----------|-----------|---------|
|                 | <i>Ppd-A1<sup>a</sup></i> | <i>Ppd-B1<sup>b</sup></i> | <i>Ppd-D1<sup>c</sup></i> | Tsukuba <sup>d</sup> |           |         | Kunneppu <sup>e</sup> |           |           |         |
|                 |                           |                           |                           | 2008/2009            | 2009/2010 | Av.     | 2007/2008             | 2008/2009 | 2009/2010 |         |
| Kitakei 221     | a                         | b                         | b                         | May 2                | May 7     | May 4   | —                     | —         | —         | —       |
| Kitakei 497     | a                         | b                         | b                         | Apr. 22              | May 1     | Apr. 26 | —                     | —         | —         | —       |
| Kitakei 1354    | a                         | b                         | b                         | May 1                | May 6     | May 3   | June 14               | June 10   | June 15   | June 13 |
| Kitakei 1463    | a                         | b                         | b                         | May 3                | May 10    | May 6   | June 13               | June 11   | June 16   | June 13 |
| Kitami 19       | a                         | b                         | b                         | May 2                | May 7     | May 4   | June 13               | June 9    | June 15   | June 12 |
| Kitami 33       | a                         | b                         | b                         | Apr. 22              | May 1     | Apr. 26 | June 9                | June 4    | June 12   | June 8  |
| Takunekomugi    | a                         | b                         | b                         | Apr. 22              | May 2     | Apr. 27 | June 6                | June 1    | June 8    | June 5  |
| Chihokukomugi   | a                         | b                         | b                         | May 1                | May 8     | May 4   | June 13               | June 10   | June 17   | June 13 |
| Kitamoe         | a                         | b                         | b                         | Apr. 30              | May 6     | May 3   | June 12               | June 10   | June 15   | June 12 |
| Kitahonami      | a                         | b                         | b                         | Apr. 29              | May 5     | May 2   | June 11               | June 8    | June 14   | June 11 |
| Kitakei 1093    | b                         | b                         | a                         | May 2                | May 8     | May 5   | June 12               | June 12   | June 17   | June 13 |
| Kitami 27       | b                         | b                         | a                         | Apr. 22              | May 2     | Apr. 27 | June 10               | June 5    | June 13   | June 9  |
| Kitami 35       | b                         | b                         | a                         | Apr. 20              | Apr. 29   | Apr. 24 | June 6                | June 4    | June 12   | June 7  |
| Tohoku 118      | b                         | b                         | a                         | Apr. 19              | Apr. 28   | Apr. 23 | —                     | —         | —         | —       |
| Horoshirikomugi | b                         | b                         | a                         | May 2                | May 8     | May 5   | June 14               | June 11   | June 16   | June 13 |
| Kitakei 320     | b                         | b                         | b                         | May 2                | May 8     | May 5   | —                     | —         | —         | —       |
| Kitakei 1409    | b                         | b                         | b                         | May 7                | May 12    | May 9   | June 14               | June 11   | June 15   | June 13 |
| Kitakei 1660    | b                         | b                         | b                         | May 8                | May 15    | May 11  | June 17               | June 13   | June 18   | June 16 |
| Kitami 18       | b                         | b                         | b                         | May 6                | May 16    | May 11  | —                     | —         | —         | —       |
| Kitami 53       | b                         | b                         | b                         | May 10               | May 18    | May 14  | June 17               | June 12   | June 17   | June 15 |
| Hokuei          | b                         | b                         | b                         | —                    | —         | —       | June 16               | June 11   | June 17   | June 14 |
| Mukakomugi      | b                         | b                         | b                         | —                    | —         | —       | June 11               | June 9    | June 15   | June 11 |
| Hokushin        | b                         | b                         | b                         | Apr. 29              | May 5     | May 2   | June 10               | June 7    | June 13   | June 10 |

<sup>a</sup> “a” and “b” indicate *Ppd-A1a* and *Ppd-A1b*, respectively.<sup>b</sup> “a” and “b” indicate *Ppd-B1a* and *Ppd-B1b*, respectively.<sup>c</sup> “a” and “b” indicate *Ppd-D1a* and *Ppd-D1b*, respectively.<sup>d</sup> Heading date at Tsukuba, 21 cultivars were tested; ‘Hokuei’ and ‘Mukakomugi’ were not tested.<sup>e</sup> Heading date at Kunneppu, 18 cultivars were tested; ‘Kitakei 221’, ‘Kitakei 497’, ‘Tohoku 118’, ‘Kitakei 320’ and ‘Kitami 18’ were not tested.

the *Ppd-A1a/Ppd-B1b/Ppd-D1b*, *Ppd-A1b/Ppd-B1b/Ppd-D1a* and *Ppd-A1b/Ppd-B1b/Ppd-D1b* genotypes was 28.4 April, 25.0 April and 5.3 May, respectively, for the 2008/2009 season. A similar genotypic difference was observed for the 2009/2010 seasons, indicating that the *Ppd-A1b/Ppd-B1b/Ppd-D1b* genotype headed 6.9–9.8 days later than other genotypes with a photoperiod-insensitive allele. Among the genotypes with a photoperiod-insensitive allele, the *Ppd-A1a/Ppd-B1b/Ppd-D1b* genotype headed 2–3 days later than the *Ppd-A1b/Ppd-B1b/Ppd-D1a* genotype, although the difference was not significant. In Kunneppu, the *Ppd-A1b/Ppd-B1b/Ppd-D1b* genotype headed 2.5 days later than other genotypes with a photoperiod-insensitive allele, although the difference was less than in Tsukuba.

As shown in Fig. 1B, monthly average temperature differed among the three wheat-growing seasons. In Tsukuba, compared with the average temperatures over the last 30 years, temperatures in winter were slightly higher during the 2008/2009 and 2009/2010 seasons. In the 2009/2010 season, temperatures were lower in April, which is just before heading. According to meteorological data from Sakaino, near Kunneppu, and the end of continuous snow cover of the test field in Kunneppu, in the 2007/2008 season,

snow melted very early and then temperatures were warmer during March and April. In the 2008/2009 season, the temperature after snow melted was warmer until heading time. In contrast, the temperature after snow melted was lower until 2 or 4 weeks before heading time in the 2009/2010 season. These differences in temperature conditions resulted in a significance difference in heading time between test years.

The *Ppd-1* genotypes of wheat cultivars and breeding lines in the pedigree of Hokkaido winter wheat cultivars are summarized in Fig. 2. Pedigree analysis showed that *Ppd-A1a* in the three cultivars, ‘Takunekomugi’, ‘Kitamoe’ and ‘Kitahonami’ was inherited from ‘Purple Straw’ through ‘Purcam (U-11)’ and ‘Hokkai 240’. *Ppd-D1a* was inherited from ‘Tohoku 118’ to ‘Kitami 27’ and ‘Kitami 35.’ However, the source of *Ppd-A1a* of ‘Kitami 19’ could not be identified.

## Discussion

The combination of *Ppd-1* alleles is important for control of the photoperiod response and hence, for the fine tuning of heading time. The introduction of photoperiod-insensitive alleles of *Ppd-1* is indispensable for breeding early-heading

**Table 4.** Effect of the *Ppd-1* genotype on the heading time in cultivars on the pedigree of 'Kitahonami'

| Growing season    | <i>Ppd</i> genotype            | Tsukuba |                   | Kunneppu |                   |
|-------------------|--------------------------------|---------|-------------------|----------|-------------------|
|                   |                                | n       | Heading date      | n        | Heading date      |
| 2007/2008         | <i>Ppd-A1a/Ppd-B1b/Ppd-D1b</i> | —       | —                 | 8        | 11.4 ± 0.9        |
|                   | <i>Ppd-A1b/Ppd-B1b/Ppd-D1a</i> |         |                   | 4        | 10.5 ± 1.7        |
|                   | <i>Ppd-A1b/Ppd-B1b/Ppd-D1b</i> |         |                   | 6        | 14.2 ± 1.2        |
| 2008/2009         | <i>Ppd-A1a/Ppd-B1b/Ppd-D1b</i> | 10      | 28.4 ± 1.4        | 8        | 7.9 ± 1.2         |
|                   | <i>Ppd-A1b/Ppd-B1b/Ppd-D1a</i> | 5       | 25.0 ± 2.9        | 4        | 8.0 ± 2.0         |
|                   | <i>Ppd-A1b/Ppd-B1b/Ppd-D1b</i> | 6       | 35.3 ± 1.7        | 6        | 10.5 ± 0.9        |
| 2009/2010         | <i>Ppd-A1a/Ppd-B1b/Ppd-D1b</i> | 10      | 35.3 ± 0.9        | 8        | 14.0 ± 1.0        |
|                   | <i>Ppd-A1b/Ppd-B1b/Ppd-D1a</i> | 5       | 33.0 ± 2.1        | 4        | 14.5 ± 1.2        |
|                   | <i>Ppd-A1b/Ppd-B1b/Ppd-D1b</i> | 6       | 42.3 ± 2.0        | 6        | 15.8 ± 0.7        |
| Average           | Growing season                 |         |                   |          |                   |
|                   | 2007/2008                      |         | —                 |          | 12.0 <sup>b</sup> |
|                   | 2008/2009                      |         | 29.6              |          | 8.8 <sup>a</sup>  |
|                   | 2009/2010                      |         | 36.9              |          | 14.8 <sup>c</sup> |
|                   | <i>Ppd</i> genotype            |         |                   |          |                   |
|                   | <i>Ppd-A1a/Ppd-B1b/Ppd-D1b</i> |         | 31.9 <sup>a</sup> |          | 11.0 <sup>a</sup> |
|                   | <i>Ppd-A1b/Ppd-B1b/Ppd-D1a</i> |         | 29.0 <sup>a</sup> |          | 11.0 <sup>a</sup> |
|                   | <i>Ppd-A1b/Ppd-B1b/Ppd-D1b</i> |         | 38.8 <sup>b</sup> |          | 13.5 <sup>b</sup> |
| F-value of ANOVA  |                                |         |                   |          |                   |
| Growing season(A) |                                |         | 25.03***          |          | 17.57***          |
| genotype(B)       |                                |         | 14.47***          |          | 4.228*            |
| (A)*(B)           |                                |         | 0.053 ns          |          | 0.201 ns          |

Heading date shows average duration (days ± standard error).

In Tsukuba, days from March 31 to heading date.

In Kunneppu, days from May 31 to heading date.

Average values of each growing season and *Ppd* genotype followed by different letters are significantly different ( $P < 0.05$ ) according to the LSD tests.

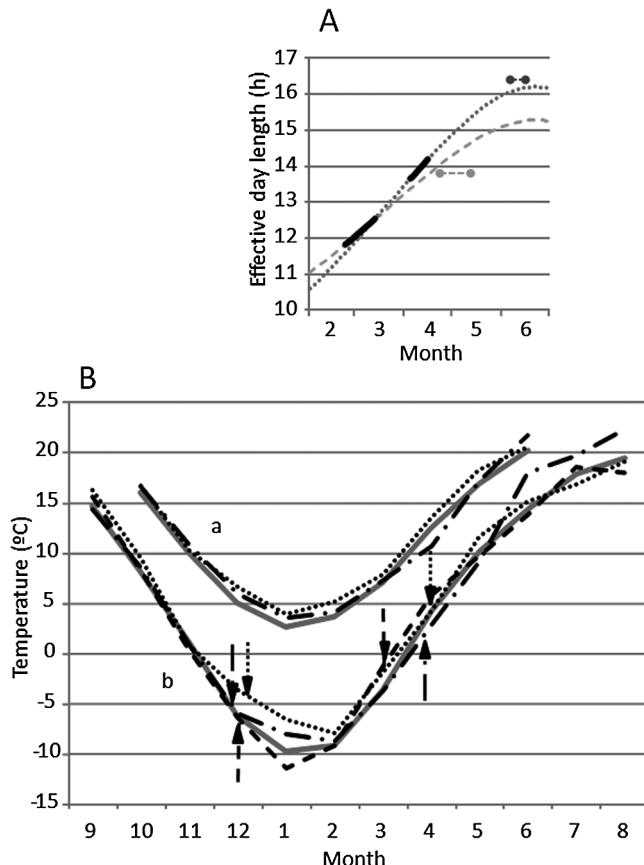
\* and \*\*\* indicate significance at the 5% and 0.1% level, respectively; 'ns' indicates no significance at the 5% level.

wheat cultivars that enable stable wheat production in Japan. To investigate the *Ppd-1* genotypes of Japanese wheat cultivars using Japanese cultivars, including 164 commercial cultivars, 24 landraces and 52 breeding lines, the *Ppd-B1* and *Ppd-D1* genotypes were determined in a previous study (Seki *et al.* 2011); the *Ppd-A1* genotype was analyzed in the present study.

Most Tohoku-Kyushu cultivars (97.5%) carried *Ppd-D1a*; extra-early cultivars (5.0%) carried *Ppd-B1a* as previously reported (Seki *et al.* 2011). However, no cultivars carried *Ppd-A1a* (Table 1). In contrast, among Hokkaido cultivars, none of the cultivars carried *Ppd-B1a* and the frequency of the *Ppd-A1a* and *Ppd-D1a* alleles generally differed between winter wheat and spring wheat. Among winter wheat cultivars, 41.4% and 24.1% carried *Ppd-A1a* and *Ppd-D1a*, respectively (Table 1). In contrast, in spring wheat cultivars, most (90%) did not carry photoperiod-insensitive alleles (Table 1).

As shown in a previous study (Seki *et al.* 2011), in Tohoku-Kyushu cultivars, heading times differed depending on their *Ppd-1* genotypes, i.e., cultivars without photoperiod-insensitive alleles headed late, those with *Ppd-D1a* headed at early to intermediate times, while those with *Ppd-B1a* and *Ppd-D1a* headed very early. Consequently, the importance of the photoperiod response in controlling heading time was

confirmed, indicating the possibility of adjusting the heading time by altering *Ppd-1* genotypes in the Tohoku-Kyushu region. In contrast, in Hokkaido cultivars, an effect of *Ppd-D1a* on heading time was not observed, indicating the involvement of other factors in controlling heading time. In this study, the heading date was investigated in Tsukuba in the Kanto region and in Kunneppu in the Hokkaido region using 23 Japanese cultivars and lines in the pedigree 'Kitahonami'. The heading date was significantly different between the *Ppd-1* genotypes (Table 4), indicating that acceleration of heading due to the photoperiod-insensitive alleles *Ppd-A1a* or *Ppd-D1a* and the effect of *Ppd-1* was lower in Kunneppu than in Tsukuba (Table 4). According to Gotoh (1977), the effective day-length at the spike formation stage, two months before heading, is approximately 14 h in Hokkaido winter wheat and 15 h in Hokkaido spring wheat, while it is shorter (approximately 12 h) in the southwestern region, which includes Kanto. Based on these data, Gotoh (1977) suggested that photoperiod sensitivity is less important for the earliness of heading in the Hokkaido region compared to in the southwestern region. Analysis of photoperiod-sensitive and -insensitive wheat cultivars clearly showed marked heading time differences between the two types under 8–12 h day-length, while the difference was small or negligible under 14–24 h day-length (Evans 1987, Klaimi



**Fig. 1.** Meteorological data in test seasons. A. Effective day-length at two sites. — : Tsukuba, ..... : Kunneppu. Lines flanked by circles indicate the range of heading date at each site. Solid and thick lines indicate the effective day-length two months before heading. B. Monthly mean temperature. — : Average temperature over the last 30 years, — : 2007/2008 growing season, ..... : 2008/2009 growing season, - - - : 2009/2010 growing season. a: Tateno (in Tsukuba), b: Sakaino (near Kunneppu). Data were taken from the Japan Meteorological Agency. Arrows indicating the start and end of continuous snow cover duration during each season in Kunneppu.

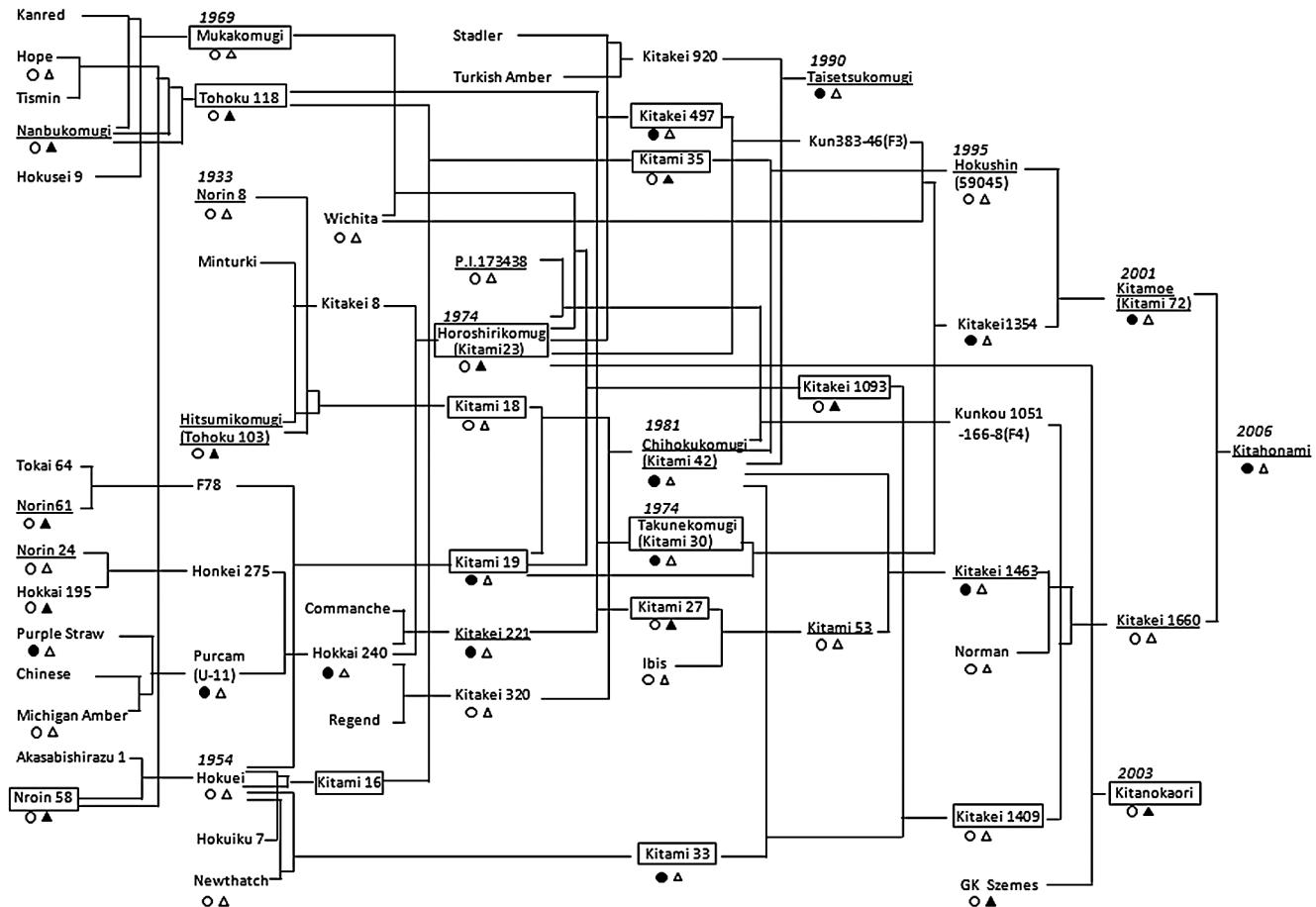
and Qualset 1973, Ormrod 1963, Slafer and Rawson 1996). These results also support the conclusion of Gotoh (1977). In this study, effective day-length two months before heading of cultivars of the pedigree ‘Kitahonami’ was approximately 14 h in Kunneppu, similar to that shown by Gotoh (1977) (Fig. 1A). Therefore, the regional difference in the day-length could explain the geographical difference of the effect of *Ppd-1* on the heading time.

Pedigree analysis of Hokkaido winter wheat cultivars suggested that *Ppd-D1a* in Hokkaido cultivars has been introduced from several Tohoku cultivars, among which ‘Tohoku 118’ is thought to be one of the donors (Fig. 2). In Hokkaido, artificial cross-breeding of wheat has been performed since 1919 to develop hard wheat cultivars. Tohoku cultivars were used as cross-parents as well as foreign cultivars, resulting in the introduction of the *Ppd-D1a* allele. For *Ppd-A1a*, pedigree analysis showed that ‘Hokkai 240’ inherited the allele from a US wheat cultivar ‘Purcam (U-11)’, for

which *Ppd-A1a* could be traced back to an old US cultivar ‘Purple Straw’ (Fig. 2). ‘Hokkai 240’, which is tolerant to leaf rust and lodging (Iriki *et al.* 1985), should have contributed to the introduction of *Ppd-A1a* into Hokkaido cultivars. Since the 1980s, soft wheat cultivars suitable for Japanese noodles have been developed in Hokkaido. Thereafter, no commercial cultivar with *Ppd-D1a* has been developed except for ‘Kitanokaori’, a hard wheat cultivar for bread. ‘Chihokukomugi’, a soft wheat cultivar with *Ppd-A1a*, was registered in 1981. This cultivar possesses high quality for Japanese noodles and is frequently crossed in wheat breeding. The shift of the breeding target from hard wheat to soft wheat was considered the turning point and then the frequency of *Ppd-A1a* increased in Hokkaido cultivars.

Hokkaido cultivars carried insensitive alleles of *Ppd-1*, although the photoperiod-insensitive alleles have less of an effect on heading time under long day conditions. A possible explanation is the pleiotropic effects of *Ppd-D1a* detected under the growing conditions in Europe and high latitude areas such as Hokkaido (Börner *et al.* 1993, Worland 1996, Worland *et al.* 1988). According to these reports, the *Ppd-D1a* allele reduced the number of spikelets per ear, resulting in increased spikelet fertility. These studies demonstrated that the photoperiod-insensitive allele *Ppd-D1a* is advantageous for increasing grain yield. The effect of *Ppd-1* on the growth pattern and grain yield in the Hokkaido region should be investigated to clarify the reason for the higher frequency of photoperiod-insensitive alleles in recently developed Hokkaido cultivars. Another possibility could be the genetic linkage between *Ppd-1* and other genes. Tolerance to lodging and diseases, particularly leaf rust, has been one of the main targets since the beginning of wheat breeding in Hokkaido. According to previous reports, the *Ppd-D1a* allele reduced plant height independently of the semi-dwarf gene *Rht* (Börner *et al.* 1993, Worland *et al.* 1988). We cannot exclude the possibility that the introduction of the lodging tolerance from ‘Hokkai 240’ resulted in the introduction of *Ppd-A1a* into Hokkaido cultivars. In contrast, no previous research has shown a close linkage between *Ppd-1* and genes for disease tolerance or flour quality, while some QTLs were reported in a homoeologous group of two chromosomes on which *Ppd-1* loci are located (Chhuneja *et al.* 2006, Roncallo *et al.* 2012, Watanabe *et al.* 2006). Additionally, studies examining the relationship between *Ppd-1* and improvement of tolerance to lodging and diseases or flour qualities in wheat breeding will reveal details of *Ppd-A1a* introduction into Hokkaido cultivars.

In this study, no Tohoku-Kyushu cultivar carried *Ppd-A1a*. Cultivars carrying *Ppd-A1a*, such as ‘Chihokukomugi’ and ‘Kitamoe’, have been used as cross parents in Tohoku-Kyushu wheat breeding; however, these cultivars carried genetic factors of late heading such as vernalization genes, so that only one cultivar, ‘Nebarigoshi’, was developed successfully in Tohoku. Introduction of *Ppd-A1a* into Tohoku-Kyushu cultivars has not been carried out so far. The photoperiod-insensitive allele *Ppd-A1a* analyzed in this study



**Fig. 2.** *Ppd-1* genotypes of wheat cultivars in the pedigree of Hokkaido winter wheat cultivars. ●: Carrier of *Ppd-A1a*. ○: Carrier of *Ppd-A1b*. ▲: Carrier of *Ppd-D1a*. △: Carrier of *Ppd-D1b*. Cultivars with no symbols were not tested for genotyping of *Ppd-1*. Some cultivars were tested for genotyping of *Pna* and *Pinb* using the method of 'Marker assist selection in wheat' project (<http://maswheat.ucdavis.edu/protocols/Hardness/index.htm>). In a previous report, Japanese hard wheat cultivars were classified as one of three genotypes, *Pna-D1b/Pinb-D1a*, *Pna-D1a/Pinb-D1b* and *Pna-D1a/Pinb-D1c* (Ikeda *et al.* 2005). In this pedigree, cultivars enclosed rectangles were *Pna-D1a/Pinb-D1b* or *Pna-D1a/Pinb-D1c*, showing these hard wheat cultivars. Cultivars with underlines were not the three genotypes classified as Japanese hard wheat cultivars, suggested that these cultivars were soft wheat. The year of registration is shown in italics.

was first found by Nishida *et al.* (2012), and thus the effect of this allele on heading time has not been extensively investigated. According to Nishida *et al.* (2012), who analyzed the heading time of a DH population segregating for three *Ppd-1* genes, the *Ppd-A1a/Ppd-B1b/Ppd-D1b* genotype headed two days later in Okayama, Chugoku region, compared to the *Ppd-A1b/Ppd-B1a/Ppd-D1b* or the *Ppd-A1b/Ppd-B1b/Ppd-D1a* genotypes, although the difference was not significant. Consistent with these data, our results demonstrate that cultivars carrying *Ppd-A1a* headed 2–3 days later in the Kanto region than those carrying *Ppd-D1a*, although the difference was insignificant (Table 4). Although early heading is important to avoid various damage in the rainy season, shorter growth periods generally result in lower grain yields; early-heading cultivars with early apical development and stem elongation are prone to frost injury (Hukumoto and Takahashi 1950, Taya 1993). Thus, heading characteristics must be adjusted for the stable production of wheat in each area. *Ppd-A1a* may be useful as a unique gene source for fine

tuning the heading time in the Tohoku-Kyushu region. Nishida *et al.* (2012) developed DNA markers to determine *Ppd-A1* genotypes, making it possible to introduce *Ppd-A1a* into Tohoku-Kyushu cultivars by MAS. However, the interaction between *Ppd-A1a* and other photoperiod-insensitive alleles is not clear. Furthermore, Eagles *et al.* (2010) indicated that the effect of *Ppd-D1a* depended on the genotype of vernalization genes. The additive effects and the interactions between *Ppd-1* genes and those between the *Ppd-1* and *Vrn-1* genes should be investigated to elucidate the usefulness of *Ppd-A1a*.

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